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ACTIVATING MUTATIONS OF THE STIMULATORY G PROTEIN IN THE McCUNE–ALBRIGHT SYNDROME

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Abstract *Background.* The McCune–Albright syndrome is a sporadic disease characterized by polyostotic fibrous dysplasia, café au lait spots, sexual precocity, and hyperfunction of multiple endocrine glands. These manifestations may be explained by a somatic mutation in affected tissues that results in activation of the signal-transduction pathway generating cyclic AMP (cAMP). We analyzed DNA from tissues of patients with the McCune–Albright syndrome for the presence of activating mutations of the gene for the α subunit of the G protein ($G_s\alpha$) that stimulates cAMP formation.

Methods. Genomic DNA fragments encompassing regions (exons 8 and 9) previously found to contain activating missense mutations of the $G_s\alpha$ gene (*gsp* mutations) in sporadically occurring pituitary tumors were amplified in tissues from four patients with the McCune–Albright syndrome by the polymerase chain reaction. The amplified DNA was analyzed for mutations by denaturing gradient gel electrophoresis and allele-specific oligonucleotide hybridization.

Results. We detected one of two activating mutations within exon 8 of the $G_s\alpha$ gene in tissues from all four patients, including affected endocrine organs (gonads, adrenal glands, thyroid, and pituitary) and tissues not classically involved in the McCune–Albright syndrome. In two of the patients histidine was substituted for arginine at position 201 of $G_s\alpha$, and in the other two patients cysteine was substituted for the same arginine residue. In each patient the proportion of cells affected varied from tissue to tissue. In two endocrine organs, the highest proportion of mutant alleles was found in regions of abnormal cell proliferation.

Conclusions. Mutations within exon 8 of the $G_s\alpha$ gene that result in increased activity of the G_s protein and increased cAMP formation are present in various tissues of patients with the McCune–Albright syndrome. Somatic mutation of this gene early in embryogenesis could result in the mosaic population of normal and mutant-bearing tissues that may underlie the clinical manifestations of this disease. (N Engl J Med 1991;325:1688-95.)

THE McCune–Albright syndrome, a disease of unknown cause, is characterized by polyostotic fibrous dysplasia, café au lait pigmentation of the skin, and multiple endocrinopathies, including sexual precocity, hyperthyroidism, pituitary adenomas secreting growth hormone, and autonomous adrenal hyperplasia.[1-4] Because of the sporadic occurrence of the McCune–Albright syndrome and the pattern of the cutaneous hyperpigmentation, it has been postulated that this disorder is due to a dominant somatic mutation occurring early in development. The early mutational event would result in a mosaic population of cells, those with and those without the mutation. The occurrence and severity of bone, skin, and endocrine abnormalities in a specific patient would depend on the number and location of cells bearing the mutation.

The endocrine glands that are hyperactive in the McCune–Albright syndrome have autonomous function.[4-9] One explanation for the manifestations of the disease is that affected tissues have an overactive cyclic AMP (cAMP) signaling pathway, which is known to stimulate the growth and function of the gonads, thyroid, adrenal cortex, specific pituitary-cell populations, melanocytes, and osteoblasts.[4,6,10-12] Possible derangements include abnormalities in components of the signal-transduction pathways that control the production of cAMP (adenylate cyclase, the stimulatory G [guanine nucleotide–binding] protein G_s, and the inhibitory G protein G_i), enzymes that degrade cAMP (phosphodiesterases), or downstream effectors (such as cAMP-dependent protein kinase).

The G proteins involved in signal transduction are heterotrimers consisting of α, β, and γ subunits, each of which is the product of separate genes.[13-15] Each G protein is defined by its α subunit, which binds guanine nucleotide and interacts with specific receptors and effectors. The activation of G proteins normally requires the interaction of the inactive guanosine diphosphate (GDP)–bound heterotrimer with the ligand-occupied receptor, resulting in the exchange of guanosine triphosphate (GTP) for GDP and dissociation of the α subunit. In the case of G_s, the GTP-bound α subunit interacts with and stimulates adenylate cyclase and specific ion channels.[15] The intrinsic GTPase activity of the α subunit inactivates the G protein by hydrolyzing the bound GTP to GDP.[14] Modifications that inhibit the GTPase activity of the α subunit of G_s ($G_s\alpha$) result in increased adenylate cyclase activity in the absence of stimulatory hormone. The addition of an adenosine diphosphate–ribose group to a specific amino acid residue (Arg^{201}) of $G_s\alpha$ by the exotoxin of *Vibrio cholerae* is one such modification.[15] Substitution of the amino acid residues Arg^{201} or Gln^{227} also results in constitutive activation of $G_s\alpha$.[16-19]

Modifications of $G_s\alpha$ can produce marked effects in vivo. Somatic mutations of Arg^{201} or Gln^{227}, referred to as *gsp* mutations, have been identified in human growth hormone–secreting pituitary adenomas and thyroid tumors.[16,20-22] Both human growth hormone–secreting pituitary adenomas and thyroid nodules are associated with the McCune–Albright syndrome.[3,4] Targeted expression of the catalytic subunit of cholera toxin produces pituitary somatotroph hyperplasia and

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gigantism in transgenic mice, presumably through the constitutive activation of G_s.[23] In Albright's hereditary osteodystrophy, an inherited deficiency of G_s is associated with multiple phenotypic abnormalities, including generalized hormone resistance.[24,25]

The features of the McCune–Albright syndrome are consistent with an early somatic mutation of the $G_s\alpha$ gene and the expression of an activated G_s protein in multiple tissues. In this study, we amplified genomic DNA fragments encompassing the sites of known activating missense mutations of $G_s\alpha$ (Arg^{201} in exon 8 and Gln^{227} in exon 9) from tissues from patients with the McCune–Albright syndrome by the polymerase chain reaction (PCR) and screened for mutations using denaturing gradient gel electrophoresis (DGGE) and allele-specific oligonucleotide hybridization. Using this approach we identified activating somatic mutations of $G_s\alpha$ in tissues from four patients with the McCune–Albright syndrome.

METHODS

Patients

The four patients studied had the classic features of the McCune–Albright syndrome, including sexual precocity, café au lait pigmentation, and polyostotic fibrous dysplasia. Multiple tissue samples, obtained surgically or at autopsy, were studied in all four patients.

Patient 1 was a three-year-old girl with mild hyperthyroidism, growth retardation, developmental delay, and chronic unexplained elevation of serum hepatocellular enzyme levels. She was born at 39 weeks of gestation weighing 2.1 kg and measuring 47 cm in length. She had transient direct hyperbilirubinemia that resolved by the third week of life, but elevations of serum hepatocellular enzymes persisted. At the age of one year she had a serum aspartate aminotransferase level of 329 U per liter, an alanine aminotransferase level of 324 U per liter, and a γ-glutamyltransferase level of 1113 U per liter; a liver biopsy showed only mild central lobular congestion. She was subsequently evaluated at the National Institute of Child Health and Human Development, Bethesda, Maryland. Her serum estrogen concentrations were elevated, and serum gonadotropin responses to luteinizing hormone–releasing hormone were abnormally low on two occasions, indicating autonomous ovarian function. Because of persistent heavy vaginal bleeding, progressive advance of bone age, and an unsatisfactory response to testolactone, an inhibitor of estrogen synthesis, the patient underwent bilateral ovariectomy at the age of two years and five months. Pathological examination of the ovaries showed thickening of the capsule, multiple follicles at all stages of development — some cystically dilated — and areas of luteinized stroma. Analysis of a liver biopsy specimen obtained during surgery revealed mild, nonspecific changes. Frozen and paraffin-embedded specimens from both ovaries, paraffin-embedded tissue from the second liver biopsy, and a blood sample were examined.

Patient 2 was a severely affected boy with hypercortisolism, goiter, pituitary adenoma, and cardiopulmonary disease who died suddenly at the age of 17.[26] The clinical history of Patient 3, a severely affected boy with hypercortisolism, hyperthyroidism, and hypersecretion of growth hormone, has been described previously.[4] This patient had unexplained cardiac arrest and died at the age of five after orthopedic surgery. The thyroid and pituitary were not available for analysis. The clinical history of and pathological findings in Patient 4, a girl with congenital Cushing's syndrome, have been described elsewhere.[5] The adrenal glands were not available for analysis.

DNA Preparation

DNA was isolated from paraffin-embedded specimens,[20] blood,[27] or frozen tissue.[28] Slides stained with hematoxylin and eosin were used to identify regions of interest in thin, paraffin-embedded sections. The regions were then scraped into tubes and processed. DNA isolated from paraffin-embedded normal tissues was used as a negative-control DNA. DNA samples from pituitary tumors with known heterozygous $G_s\alpha$-activating mutations, kindly provided by J. Lyons, were used as positive-control DNA.

PCR Amplification of Genomic DNA

Either 2 μg of crude DNA prepared from paraffin-embedded tissues or 0.1 to 1 μg of DNA from blood or frozen tissue was amplified in a 100-μl PCR[29] mixture containing deoxynucleotide triphosphates (200 μmol per liter each), upstream and downstream oligonucleotide primers (0.5 μmol per liter each), 0.01 percent (wt/vol) gelatin, 50 mmol of potassium chloride per liter, 10 mmol of TRIS–hydrochloric acid per liter (pH 8.3), 1.5 or 2.5 mmol of magnesium chloride per liter, and 2.5 U of *Taq* polymerase (Perkin–Elmer Cetus). The reactions contained 2.5 mmol of magnesium chloride per liter for exon 9 and 1.5 or 2.5 mmol per liter for exon 8. Strict precautions were taken to keep amplified DNA separate from areas in which patient samples were handled. In all PCR experiments several reactions containing no DNA were included to control for the possibility of contamination. PCR reagents were treated with ultraviolet light (254 nm) before the addition of *Taq* polymerase, mineral oil, and DNA. Amplification consisted of denaturation at 94°C for 5 minutes, followed by 35 to 45 cycles consisting of annealing at 58°C for 45 seconds, primer extension at 72°C for 1 minute, and denaturation at 94°C for 1 minute, and 1 final cycle with a 3-minute primer extension. The PCR products were analyzed on 5 percent nondenaturing acrylamide gels.

"Melting" maps (plots of the midpoint melting temperature as a function of position along a DNA sequence) were generated for genomic fragments encompassing exon 8 and exon 9 by a computer algorithm[30] and were used to determine the site of attachment of a stable, GC-rich fragment (the GC clamp).[31] The addition of a GC clamp to one end of a DNA fragment increases the ability of DGGE to detect mismatches in the sequence of interest. For exon 8 the primers were 5′TCGGTTGGCTTTGGTGAATCCA3′ (intron 7 sequence) and 5′CGCCCGCCGCGCCCCGCGCCCGTCCCGC-CGCCCCCGCCCCCAGAAACCATGATCTCTGTTATA3′ (the GC clamp was attached to the 5′ end of a complementary sequence of intron 8, which is underlined).[32] For exon 9 the primers were 5′AACTGCAGCCAGTCCCTCTGGAATAACCAG3′ (the sequence of intron 8 is underlined) and 5′CGCCCGCCGCGCCCC-GCGCCCGTCCCGCCGCCCCCGCCCCAGCGACCCTGATC-CCTAACAAC3′ (the GC clamp was attached to the 5′ end of a complementary sequence of intron 9, which is underlined).[32] Oligonucleotides were synthesized and purified as previously described.[24]

DGGE

DGGE was performed as previously described.[24,33] PCR samples (10 to 15 μl) were heated to 95°C for 5 minutes, rapidly cooled, and then subjected to electrophoresis for 15 to 20 hours at 85 V on a 7 percent acrylamide vertical gel with a parallel gradient of linearly increasing denaturing conditions. The denaturing gradients used were 35 to 65 percent for exon 8 and 35 to 90 percent for exon 9 (100 percent denaturing condition is created by 7 mol of urea per liter and 40 percent [vol/vol] formamide). Gels were stained with silver (Bio-Rad kit). Melting maps of the exon 8 and 9 fragments predicted that DGGE would detect mutations anywhere within the coding sequences.

Allele-Specific Oligonucleotide Hybridization

PCR samples (3 to 20 μl) were denatured in 0.2 *N* sodium hydroxide, 15 mmol of TRIS–hydrochloric acid per liter (pH 7.5), and 3.75 mmol of EDTA per liter for 30 minutes at room temperature and then dot blotted onto nylon filters (Oncor Sure Blot). The filters were incubated for two hours in an 80°C vacuum oven and then hybridized with oligonucleotide probes end-labeled with phosphorus-32 (20 bases in length) that contained the wild-type sequence of arginine at position 201 (R201; codon sequence, CGT), a substitution of cysteine for arginine at position 201 (R201C; codon sequence, TGT), or a substitution of histidine for arginine at position 201 (R201H; codon sequence, CAT). The oligonucleotide-probe sequences as well as hybridization and washing conditions

have been described previously.[20] Results from representative positive and negative tissues were confirmed by analyzing an independent DNA preparation from the same paraffin block.

Results

Amplification and Analysis of Exons 8 and 9

A genomic fragment of DNA of 204 base pairs (bp) encompassing exon 8 was amplified by PCR from tissue or blood samples from the four patients. Attempts to amplify a number of paraffin-embedded bone samples from Patients 2 and 3 were unsuccessful, probably because DNA degradation had occurred during acidic decalcification of these specimens.[34,35]

The PCR products were then analyzed by DGGE, a method that allows rapid screening for mutations, including single-base substitutions, in a DNA region. Double-stranded DNA fragments consisting of mismatched wild-type and mutant strands (heteroduplexes) will melt more readily than those consisting of two matched strands (homoduplexes) and will therefore migrate a shorter distance in a gradient of denaturant.[33] DNA amplified from normal tissues revealed only a wild-type homoduplex band (Fig. 1, negative control). DNA amplified from pituitary tumors known to contain either the R201C or R201H substitution revealed an abnormal pattern with two slower-migrating heteroduplex bands and a mutant homoduplex band in addition to the wild-type band (Fig. 1, positive control; data shown only for R201C). Analysis of several tissue samples from Patient 1 revealed a pattern resembling that of the pituitary tumors, with varying ratios of abnormal and wild-type bands (Fig. 1). Similar results were obtained with tissues from the three other patients (data not shown).

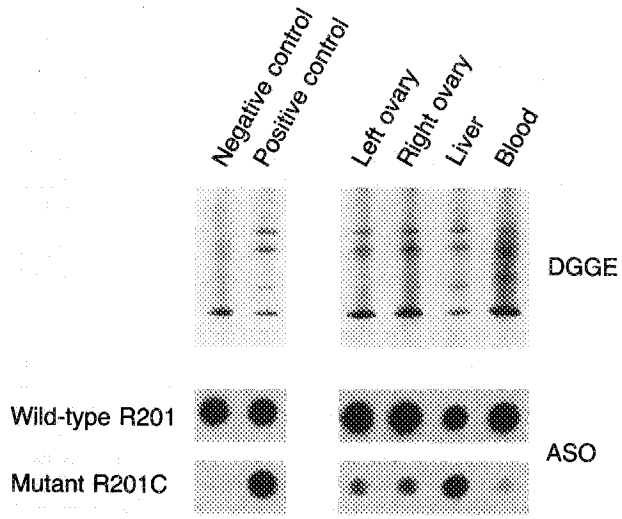

Figure 1. Analysis of Tissue Samples from Patient 1, a Negative Control, and a Positive Control.

PCR-amplified genomic fragments encompassing exon 8 from control tissues and from tissues from Patient 1 were analyzed by both DGGE and allele-specific oligonucleotide hybridization (ASO). For the latter, the DNA samples were hybridized with oligonucleotide probes containing the wild-type R201 and mutant R201C sequences. Paraffin-embedded normal adrenal tissue was used as the negative control, and a pituitary tumor known to contain the R201C mutation was used as the positive control. In the samples analyzed by DGGE, two additional upper heteroduplex bands were detected in the positive control and in all patient samples, whereas the mutant homoduplex band was seen only in the samples with the highest proportion of mutant alleles (positive control and liver).

To identify the specific mutations responsible for the abnormal patterns, the amplified DNA samples were analyzed by allele-specific oligonucleotide hybridization. In this technique DNA is bound to nylon filters and hybridized at high stringency to radiolabeled oligonucleotide probes that either match the wild-type sequence or contain specific single-base substitutions. Analysis with the wild-type R201 and mutant R201C and R201H oligonucleotide probes revealed that the DNA abnormalities revealed by DGGE in each patient could be accounted for by either one of the two previously defined activating missense mutations at Arg^{201}. Different tissues from an individual patient all had varying amounts of the same mutant allele (either R201C or R201H). Moreover, for each sample the relative proportion of DNA present in the abnormal upper bands in DGGE correlated well with the relative proportion of mutant allele determined by allele-specific oligonucleotide hybridization (Fig. 1). The results were negative with use of DNA prepared from six paraffin-embedded tissues from patients without the McCune–Albright syndrome.

Screening of a PCR-amplified 218-bp genomic fragment encompassing exon 9 from selected tissues from the four patients revealed no evidence of mutations (data not shown). In these experiments pituitary-tumor samples known to contain two different missense mutations coding for substitutions at Gln^{227} within exon 9 produced abnormal patterns.

Patient 1

Screening for mutations in exon 8 revealed the R201C mutation in frozen surgical specimens of both ovaries from Patient 1 (Fig. 1). The same mutation was also present in genomic DNA isolated from a paraffin-embedded liver specimen and from whole blood (Fig. 1). The R201C mutation was confirmed by direct sequencing of DNA amplified from liver and ovary (data not shown). An area of histologically abnormal ovarian tissue contained a higher proportion of mutant alleles than an adjacent normal area (Fig. 2). DNA isolated from a region of the paraffin-embedded sample containing cortical stroma and primordial follicles contained almost no mutant alleles, whereas that from a region of luteinized follicular cells, whose presence is abnormal in a three-year-old girl, had equally intense signals for normal and mutant sequences, suggesting that almost all cells from this area were heterozygous for the activating mutation. DNA from the whole slice, which included affected and unaffected regions, contained an intermediate

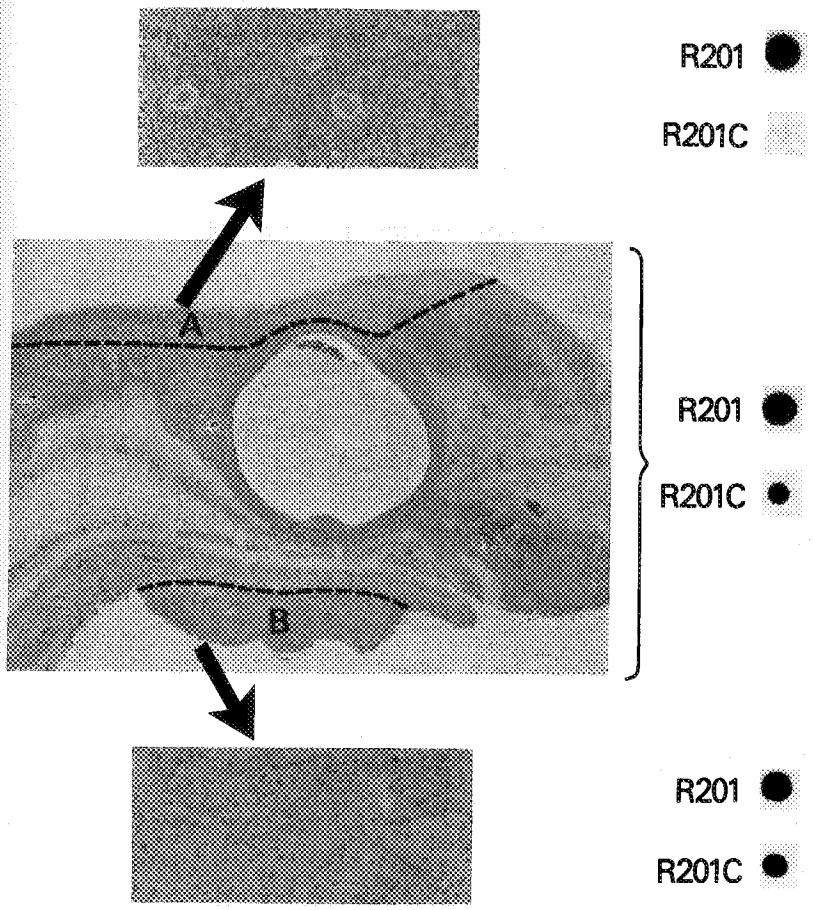

Figure 2. Correlation of the Abundance of Mutant Alleles with the Pathological Abnormalities in Ovarian Tissue from Patient 1.

A cross-section from a paraffin-embedded section of ovary from Patient 1 is shown in the center (×50). The two outlined areas, shown at higher magnification (×120), were dissected and analyzed independently; area A shows ovarian cortex containing primordial follicles, and area B follicular cyst lining containing luteinized theca. Blots showing the results of hybridization of DNA from these regions with wild-type R201 and mutant R201C oligonucleotide probes after PCR amplification for the whole ovary section, area A, and area B are shown on the right.

amount of mutant $G_s\alpha$ that corresponded to the results obtained with the frozen ovary specimens (Fig. 1). DNA isolated from skin fibroblasts derived from normal and pigmented areas did not contain the R201C mutation (data not shown).

Patient 2

The R201C mutation was found in several tissues from Patient 2 (Fig. 3), including the thyroid, pituitary adenoma, and right and left adrenal glands. Immunostaining of the pituitary adenoma for hormones was positive only for growth hormone (data not shown). The mutant allele was also detected, but in lower proportions, in testis, thymus, lung, liver, heart, kidney, and spleen; it was not detected in the parathyroid or bowel (Fig. 3). The R201C mutation was confirmed by direct sequencing of DNA amplified from the left adrenal adenoma (data not shown). The left adrenal specimen included one region of adrenocortical adenoma and another of normal adrenal tissue. When these regions were analyzed separately, the relative proportion of mutant alleles was much higher in adenomatous tissue than in the normal adrenal tissue (Fig. 3).

Patient 3

The R201H mutation was found in a number of tissue samples from Patient 3 (Fig. 4). The mutation was confirmed by direct sequencing of DNA amplified from liver (data not shown). The adrenal glands, testis, liver, and heart had the highest proportion of mutant alleles, whereas most of the other tissues examined, including gastric adenomatous polyps, thymus, lung, pancreas, kidney, and mesenteric lymph node, contained detectable but lower proportions of the mutant alleles. The mutant DNA was barely detectable in the esophagus.

Patient 4

In Patient 4 the R201H mutation was found in paraffin-embedded surgical specimens of the cystic right ovary and histologically normal right fallopian tube but not the left ovary (data not shown). Although the left ovary was reported to be histologically abnormal, the specimen available for analysis contained mostly stroma with primordial follicles, findings that perhaps explain our inability to detect the mutation in this specimen. The R201H mutant allele was also present in a low proportion in DNA amplified from a recent blood sample from this patient (data not shown).

Discussion

We identified activating mutations of the $G_s\alpha$ gene in tissues from four patients with the McCune–Albright syndrome. It has been postulated that the McCune–Albright syndrome may be caused by either an early postzygotic somatic mutation or a gametic half-chromatid mutation that results in the widespread, mosaic distribution of abnormal cells.[36] Clinical features that support this concept include the sporadic occurrence of the syndrome and the characteristic, often lateralized, pattern of skin and bone involvement. Our findings also support this model. First, in each patient only one specific mutation (R201C or R201H) was detected, a finding that is

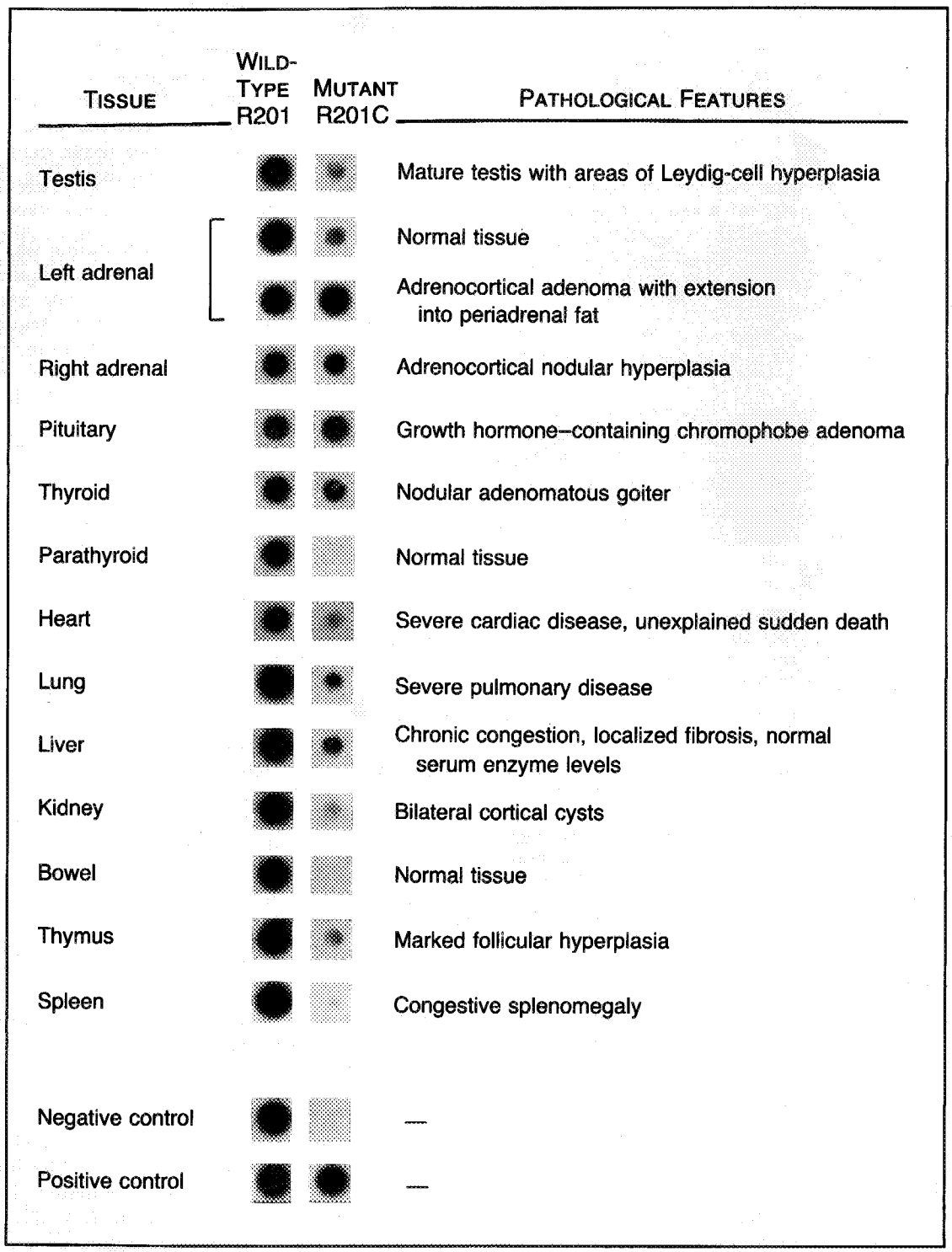

Figure 3. Analysis of Tissue Samples from Patient 2.

PCR-amplified genomic fragments encompassing exon 8 from paraffin-embedded tissues from Patient 2 and from control tissues were hybridized with the wild-type R201 and mutant R201C oligonucleotide probes. The left adrenal tissue was obtained at surgery, and the other samples were obtained at autopsy. Adjacent regions of adrenocortical adenoma and normal adrenal tissue from the same slide were dissected and analyzed independently. The pathological findings for each tissue[26] are outlined on the right. Cardiac disease included cor pulmonale and intermittent left ventricular failure. Pulmonary disease included severe restrictive disease due to kyphoscoliosis, mild obstructive disease, recurrent infections, and chronic congestion. Paraffin-embedded normal adrenal tissue was used as the negative control, and pituitary tumor known to contain the R201C mutation was used as the positive control. The results for heart and spleen are from a separate experiment with comparable controls.

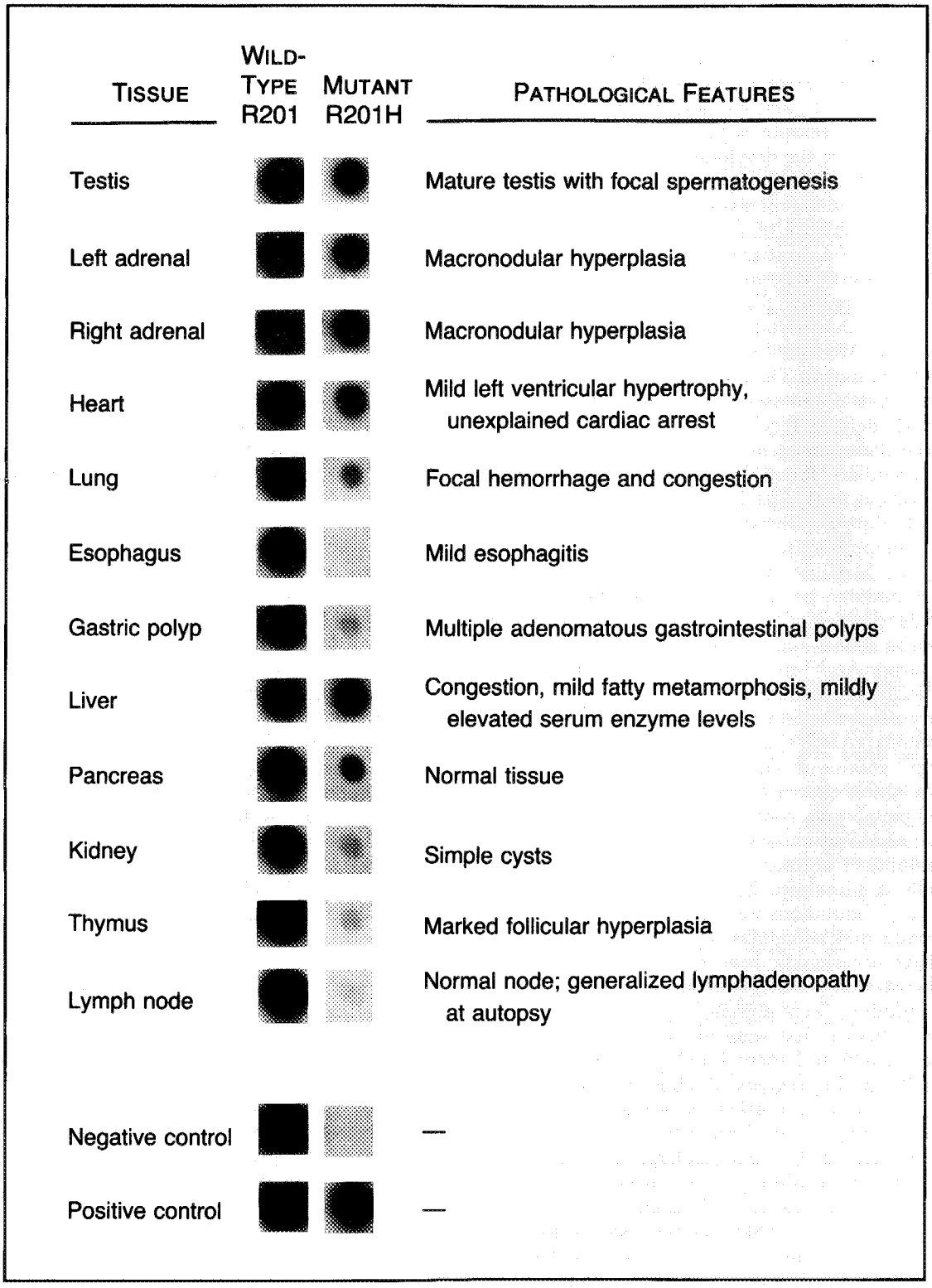

Figure 4. Analysis of Tissue Samples from Patient 3.

PCR-amplified genomic fragments encompassing exon 8 from paraffin-embedded tissues from Patient 3 were hybridized with wild-type R201 and mutant R201H oligonucleotide probes. All samples were obtained at autopsy except for the adrenal samples, which were obtained at surgery. The pathological findings for each tissue are outlined on the right. This patient had slightly elevated serum levels of hepatocellular enzymes on two occasions while he was taking propylthiouracil. Paraffin-embedded normal adrenal tissue was used as the negative control, and pituitary tumor known to contain the R201H mutation was used as the positive control.

consistent with the presence of a single monoclonal population of abnormal cells. Second, the widespread distribution of the mutation in tissues derived from all three embryologic germ layers (ectoderm [pituitary], endoderm [thyroid], and mesoderm [adrenal glands] in Patient 2, for example) suggests a mutational event that occurs before the development of the trilaminar disk. Third, the variable representation of the mutation among the different tissues in an individual patient and the absence of a mutation in at least one tissue from each patient are consistent with a somatic rather than a germ-line mutation.

Preliminary positive results in four of six other patients suggest that mutations of Arg^{201} may be common in the McCune–Albright syndrome (unpublished observations). The ability of mutant $G_s\alpha$ genes to be expressed as messenger RNA and their association with defined biochemical abnormalities have been well documented in human pituitary tumors.[16] We have similarly found that the mutant R201C gene is expressed as messenger RNA in ovarian tissue from Patient 1 (data not shown). Direct biochemical evidence of inappropriate activation of the cAMP pathway in the McCune–Albright syndrome is lacking, however, and may be difficult to obtain because of the mosaic distribution of the mutation within tissues and the need to collect appropriate control tissues.

Activating Arg^{201} mutations (*gsp* oncogenes) were first described in sporadic growth hormone–secreting pituitary adenomas, in which they are associated with autonomous cAMP synthesis.[16,20,21] Replacement of the Arg^{201} residue in $G_s\alpha$ with cysteine or histidine causes a 30-fold decrease in intrinsic GTPase activity, and cell membranes containing these altered proteins produce cAMP at an elevated rate in the absence of any stimulatory hormone.[16] The proliferation of certain cells is stimulated by cAMP.[11,12,37] In our patients, Arg^{201} mutations were associated with cellular hyperplasia and adenomas, although malignant tumors have occasionally been reported in association with the McCune–Albright syndrome.[4,10,38]

$G_s\alpha$ mutations were present in virtually all affected endocrine tissues that were analyzed. Moreover, in ovarian tissue from Patient 1 and adrenal tissue from Patient 2, specific regions of abnormal tissue contained a higher proportion of mutant cells than adjacent normal tissue. Primordial ovarian follicles containing activated G_s may undergo maturation independently of gonadotropins, resulting in the development of cystic ovaries and sexual precocity. Increased synthesis of cAMP has been shown to have acute and chronic stimulatory effects on steroidogenesis in adrenal glands and gonads.[39] Hyperplastic expansion of abnormal cells during follicular maturation would result in a region in the ovary rich in cells bearing the mutation. Proliferation of mutant cells may also account for their being more abundant in an adrenocortical adenoma than in the normal adrenal gland in Patient 2. Although the role of increased synthesis of cAMP in stimulating the proliferation of ovarian and adrenal cortical cells has been questioned,[20] the association of activating $G_s\alpha$ mutations with cellular proliferation in the McCune–Albright syndrome suggests that both gonadotropins and corticotropin may use the G_s pathway to stimulate proliferation of their respective target tissues in vivo.

The growth hormone–producing pituitary adenoma in Patient 2 had a high level of the R201C mutant allele. R201 substitutions are the most common *gsp* mutations found in pituitary tumors.[16,20,21] The transcription of growth hormone factor 1 (GHF1), a protein that promotes differentiation and proliferation of somatotrophs, is induced by cAMP.[40] Only growth hormone–secreting pituitary tumors have been described in the McCune–Albright syndrome. Somatic mutations of G_s may occur in other pituitary cell lines, but perhaps only the somatotrophs respond with uncontrolled proliferation.

Thus far, we have been unable to determine whether activating mutations are present in dysplastic bone and café au lait spots, two of the prominent characteristics of the McCune–Albright syndrome. Attempts to amplify specimens of bone and skin from several patients were unsuccessful, and cultured skin fibroblasts from Patient 1 did not harbor the activating mutation. In both dysplastic bone and café au lait spots there are very few of the potentially relevant types of cells (osteoblasts and melanocytes, respectively),[11,41] so that even in appropriately prepared specimens it may be difficult to identify mutations.

It remains to be determined whether G_s mutations are causally related to the nonendocrine abnormalities in three of our patients, including chronic liver disease (Patient 1), thymic hyperplasia (Patients 2 and 3), gastrointestinal adenomatous polyps (Patient 3), cardiopulmonary disease (Patient 2), and sudden death (Patients 2 and 3). Unexpectedly widespread pathology, including thymic hyperplasia and gastrointestinal polyps, has previously been noted,[26,38,42] but to our knowledge liver disease has not. In addition to Patient 1, Patient 2 also had evidence of mild liver abnormalities (Fig. 3). We are aware of two other severely affected patients whose histories included chronic, unexplained liver disease and unexpected cardiopulmonary arrest (Pescovitz O, Charest N, Van Wyk JJ: personal communication). Further attention should be focused on the potential clinical implications of constitutive activation of G_s in nonendocrine tissues, such as the liver and the cardiac pacemaker.[43,44]

$G_s\alpha$ mutations were detected in a few tissues (e.g., pancreas, kidney, and blood) with no clinical abnormalities. One explanation for this finding is that there is low expression of the mutant gene as messenger RNA or protein in these tissues. Another possibility is that increased cAMP production has minimal consequences in some cells, possibly because of a compensatory increase in phosphodiesterase activity.[45]

The correlation of activating $G_s\alpha$ mutations with endocrine pathology and the physiologic relevance of

constitutive cAMP production to the autonomous endocrine hyperfunction of the McCune–Albright syndrome are compelling evidence that these mutations represent the pathogenetic basis of this disease. The McCune–Albright syndrome is a potential in vivo model of the role of G_s signaling pathways in biologic systems and human disease.

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